Observation of Collagen-Containing Lesions After Hematoma Resolution in Intracerebral Hemorrhage

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Abstract: Background and purpose: The classic presentation of chronic (stage III) hemorrhagic stroke lesions is a fluid-filled cavity. In one of the most commonly used animal models of intracerebral hemorrhage (ICH), we noticed additional solid material within the chronic lesion. We examined the composition of those chronic ICH lesions and compared them with human autopsy cases. Methods: ICH was induced in rats by the injection of collagenase in the striatum. Tissue sections after hematoma resolution corresponding to 3 different chronic time points-28, 42, and 73 to 85 days post-ICH-were selected. Human autopsy reports at the University Hospital of Zurich were searched between 1990 and 2019 for ICH, and 3 chronic cases were found. The rat and human sections were stained with a variety of histopathologic markers. Results: Extensive collagenous material was observed in the chronic lesion after hematoma resolution in both the rat model and human autopsy cases. Additional immunostaining revealed that the material consisted primarily of a loose network of collagen 3 intermingled with occasional GFAP (glial fibrillary acidic protein)-positive processes and collagen 4. Conclusions: A key feature of the chronic ICH lesion is a loose network of collagen 3. The collagenase rat model reproduces the morphology and composition of the chronic human ICH lesion. While identifying new features of ICH lesion pathology, these results are important for treatment and recovery strategies.

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Observation of collagen-containing lesions after hematoma resolution in intracerebral hemorrhage

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Background and purpose—The classic presentation of chronic (Stage III) hemorrhagic stroke lesions is a fluid-filled cavity. In one of the most commonly used animal models of intracerebral hemorrhage (ICH), we noticed additional solid material within the chronic lesion. We examined the composition of those chronic ICH lesions and compared them with human autopsy cases.

Methods—ICH was induced in rats by the injection of collagenase in the striatum. Tissue sections after hematoma resolution corresponding to three different chronic time points—28, 42, and 73-85 days post-ICH—were selected. Human autopsy reports at the University Hospital of Zurich were searched between 1990 and 2019 for ICH and three chronic cases were found. The rat and human sections were stained with a variety of histopathological markers.

Results—Extensive collagenous material was observed in the chronic lesion after hematoma resolution in both the rat model and human autopsy cases. Additional immunostaining revealed that the material consisted primarily of a loose network of collagen 3 intermingled with occasional GFAP-positive processes and collagen 4.

Conclusions—A key feature of the chronic ICH lesion is a loose network of collagen 3. The collagenase rat model reproduces the morphology and composition of the chronic human ICH lesion. While identifying new features of ICH lesion pathology, these results are important for treatment and recovery strategies.

Nonstandard Abbreviations and Acronyms

ICH intracerebral hemorrhage

GFAP glial fibrillary acidic protein

NeuN neuronal nuclear protein

α-SMA alpha smooth muscle actin

H&E hematoxylin and eosin

eVG elastic Van Gieson

Introduction

The evolution of hemorrhagic stroke lesions consists of three stages: deformation, edema, and necrosis of the surrounding tissues (Stage I); resorption of the hematoma (Stage II); and full resolution of the hematoma resulting in a fluid-filled cavity (Stage III).[1] In human Stage II lesions, a capsule-like, collagenous border has been observed.[2,3] Histological examination after autologous blood injection in dogs reveals the formation of argentophilic fibers on the border of the hematoma with a gradual change to more mature collagenous fibers. However, the studies were limited to Stages I and II with the hematoma still present.[4,5] Prior reports of Stage III hemorrhagic lesions in humans describe a thin, fluid-filled cavity that is indistinguishable from a Stage III infarct except for the presence of blood-related deposits such as hemosiderin and walls and fluid that have a yellow to brown coloration.[1,2,6,7]

Histopathological characterization of chronic stroke lesions is important for the development of new biomaterial-based therapies[8] that physically interact with lesion constituents and require a space to accommodate the injection. Development of these emerging therapies typically begins in rodent stroke models, but lesion outcomes may be species dependent.[9] Therefore, histopathological comparison is essential to determine if chronic lesions in animal models reproduce the key features found in clinical cases.

While developing a biomaterial-based therapy[10] for chronic ICH in a collagenase-induced rat model[11], we noticed that the chronic rat lesions were not a simple fluid-filled cavity as expected. Herein, we report on the composition of these lesions and compare them with human autopsy cases of chronic ICH.

Methods

This study adheres to the AHA journals' implementation of the Transparency and Openness Promotion Guidelines; the authors declare that all supporting data are available within the article (and in the Data Supplement). Human autopsy reports at the University Hospital of Zurich were searched between 1990 and 2019 for ICH and three chronic cases (1 male and 2 females) were found. The Executive Director of the Kantonale Ethikkommission provided notification that the histopathologic examination of autopsy samples did not fall within the scope of the Human Research Act (HRA). Human samples consisted of formalin-fixed tissue embedded in paraffin with immunostaining for GFAP, collagen 3, collagen 4, NeuN, and α-SMA by standard methods based on the oxidation of 3,3'-diaminobenzidine.

The rat model consisted of injection of collagenase (0.1U type VII in 1μL saline) into the right striatum of male Sprague-Dawley rats (280-300 grams). All procedures were performed in accordance with the Institutional Animal Care and Use Committee of Massachusetts General Hospital (animal protocol number 2012N000165) and the Guide for Use and Care of Laboratory Animals from the National Institute of Health (NIH). At 28, 42, and 73-85 days post-ICH, the rats (n=6 per group) were sacrificed and the extracted brains were fixed in 4% paraformaldehyde, cryopreserved in 30% sucrose, and

frozen. Cryosections (30μm) were immunostained for GFAP, collagen 3, collagen 4, NeuN, and α-SMA by standard methods based on a secondary fluorescent antibody.

Additional experimental details for the human and rat samples are provided in the Data Supplement.

Results

In a well-validated, collagenase-induced rat model of ICH[11], we noticed an unexpected material inside chronic lesions after hematoma resolution (Figure 1). Further staining revealed that the material consisted primarily of collagen 3 intermingled with occasional GFAP-positive processes and collagen 4. Collagen 3 is typically associated with reticular fibers and forms the loose extracellular matrix found early within a wound that heals by coagulative necrosis. There was no significant difference over time in the amount of collagen 3 or lesion volume that would indicate wound healing (Figure I in the Data Supplement). The formation of a stable, hemorrhagic lesion in rat after hematoma resolution (~ 2-3 weeks depending on initial hematoma size) is consistent with prior studies.[12,13]

At all three examined time points and for reasons that are not yet known, we observed two types of lesion morphology: thin, compacted lesions and larger, less dense lesions. The collagenous fibers were found within both lesion types (Figure 1). NeuN (Figure II in the Data Supplement) and α-SMA (Figure III in the Data Supplement) were absent within the lesions at all time points. Negative controls are included in the Data Supplement (Figure IV).

To check our rat model observations with human outcomes, we searched autopsy reports at the University Hospital of Zurich between 1990 and 2019 for ICH and found three Stage III cases (Table and Figure V in the Data Supplement). Stage III hemorrhagic lesions were not the direct cause of death in any of the patients.

An unexpected finding was the extensive extracellular matrix (ECM) inside the lesion in the H&E stained sections (Figure 2). Subsequent elastic Van Gieson (eVG) staining (Figure 2) revealed that most of the material consisted of loosely organized collagen fibers (in pink). We performed additional immunostaining, which revealed predominantly type 3 collagen (Figure 2). GFAP, non-vascular α-SMA, and NeuN were negative within the lesions (Figure VI in the Data Supplement). The lack of pervasive α-SMA is consistent with the finding of a loose network of collagen 3 in contrast to the contracted collagen 1 scar of chronic skin and musculoskeletal lesions. Some calcification was observed in the lesion of patient 2. Negative controls are included in the Data Supplement (Figure VII).

Discussion

We observed extensive collagenous material, primarily collagen 3 with occasional GFAP-positive processes and collagen 4, in the chronic lesion after hematoma resolution in both the rat model and human autopsy cases. The source of the collagen-producing cells is unknown. Proposed sources based on brain and spinal cord lesions in rodents include meningeal fibroblasts[14], perivascular fibroblasts[15], and endothelial cells[16]. The proposed purpose of the collagen in rodent lesions is to provide a scaffold for invading fibroblasts, macrophages, and blood vessels.[14] In Stage II ICH human lesions, we observe a collagen capsule surrounding the hematoma that is distinct

from an outer GFAP-positive glial layer, which contains CD68-positive microglia/macrophages (Figure VIII in the Data Supplement). This subacute capsule consists primarily of collagen 3 (Figure VIII in the Data Supplement); therefore, the collagen 3 observed in Stage III lesions might be related to the Stage II capsule.

We were surprised to find only a few chronic cases of ICH in the archives. Perhaps chronic ICH lesions are underrepresented because incidence is lower and acute mortality is higher compared to ischemic stroke.[17] Nowadays, very few autopsies for ICH are performed because they can be diagnosed by neuroimaging. While old microbleeds are difficult to detect, we tried looking at cavernous hemangiomas, which are known to bleed repeatedly with a prolonged history; however, the distinction between collagen associated with atypical vessels and putative bleeding-associated tissue is difficult. Further studies of larger numbers of patients would be helpful to confirm our findings and expand our understanding of them—such as if they are global or restricted to ICH in specific brain regions.

Despite the very different time of investigation post-ICH of the rat (tens of days) and human Stage III lesions (years), they are found with the same morphology and composition. Our comparison is based on the end stage of the lesion, and further work is needed to determine if these findings are coincidental or related to similarities in hematoma resolution with a different time scale based on the initial hematoma size.

The examination and comparison of chronic stroke lesions in animal models and human clinical cases is important for treatment and recovery strategies. The injection of a biomaterial-based therapy into a chronic lesion requires space to accommodate the biomaterial. The loose, porous collagen 3 matrix that was observed unexpectedly in the

chronic rat lesion did not inhibit the subsequent injection of a biomaterial into the lesion.[10] Presumably, the liquid injection displaces the collagen fibers or infiltrates the porous network before gelation in situ. Based on our findings of a similar lesion composition in the human cases, we anticipate that chronic human ICH lesions can also accommodate and prove amenable to treatment with a biomaterial-based therapy.

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Disclosures. None.

Supplemental Materials

Table

Figures I - IX

Expanded Materials and Methods

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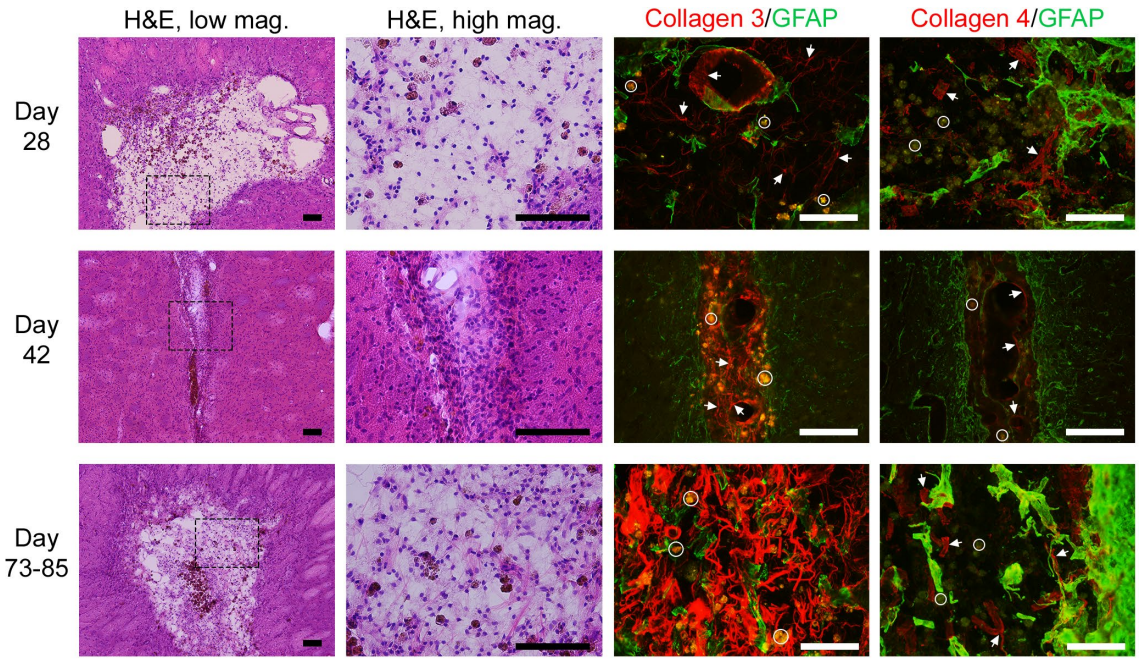
Figure Legends

Figure 1. The main constituent of chronic ICH lesions after hematoma resolution in a collagenase-induced rat model is collagen 3. Each row corresponds to a representative lesion in the striatum at 28 days (n=6), 42 days (n=6), and 73-85 days (n=6) post-ICH. Note that collagen 3 is seen in both thin, compacted lesions (42 days) as well as larger, less dense lesions (28 and 73-85 days). GFAP is predominantly observed at the lesion border. The white arrows indicate examples of structures of interest, and the white circles indicate examples of autofluorescence from hemosiderin. H&E scale bars (black) indicate 50μm and fluorescent image scale bars (white) indicate 100μm. For additional details on the lesion location, see Figure IX in the Data Supplement.

Figure 2. The lesion in chronic human ICH consists primarily of collagen 3. Each column corresponds to the indicated patient number (for additional case and location details, see the Table and Figure V in the Data Supplement). Each row is labeled by its corresponding marker. The top row provides an overview of the three chronic ICH lesions at low magnification followed by high magnification images that correspond to the dashed boxes. Elastic Van Gieson (eVG) staining at high magnification highlights (in pink) the extensive collagen found inside the lesions. Additional staining with collagen 3 and 4 reveals that the lesion consists primarily of collagen 3. All unlabeled scale bars after the first row indicate 100μm.



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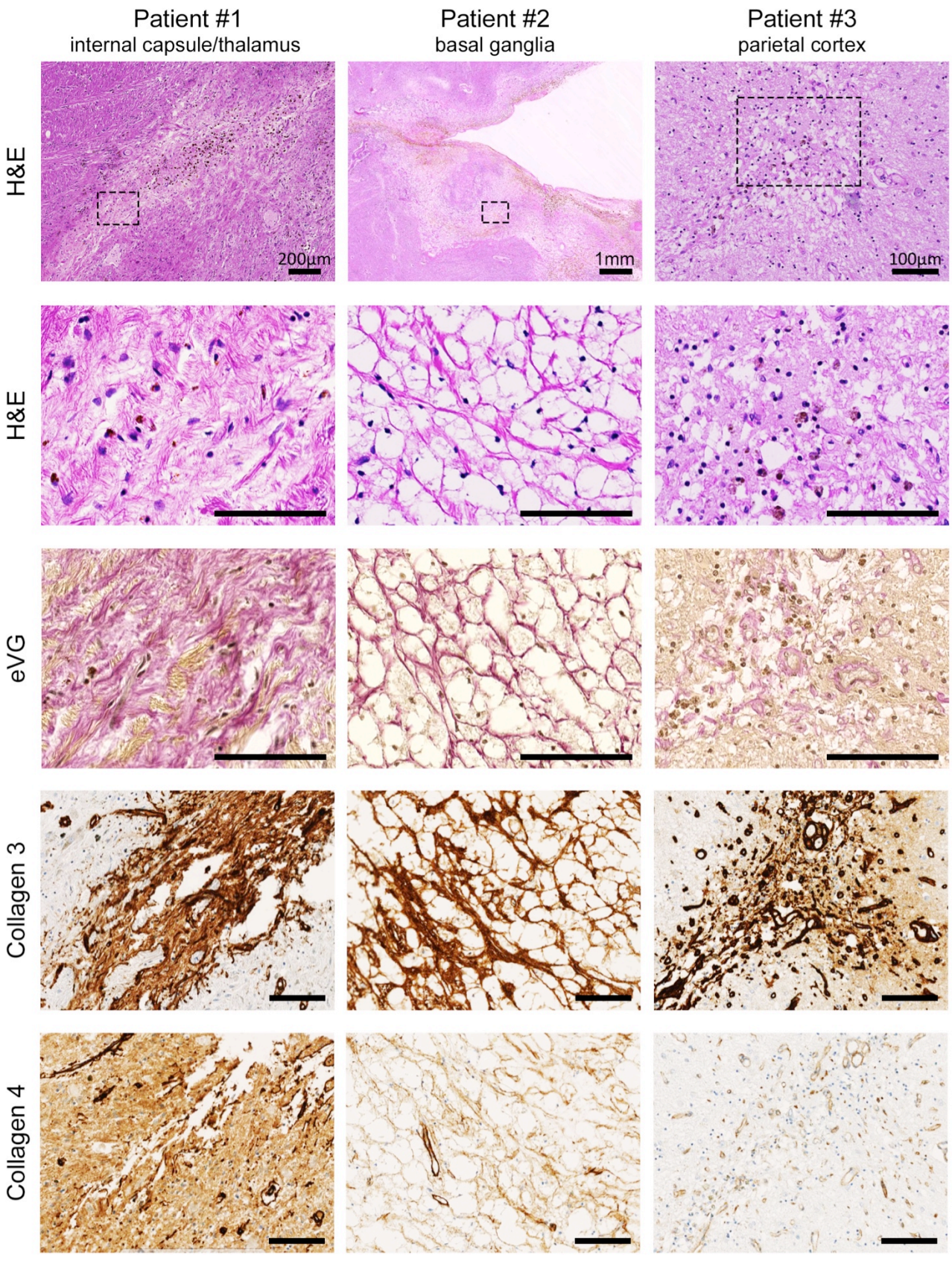
Patient #1
internal capsule/thalamus
Patient #2
basal ganglia
Patient #3
parietal cortex
H&E
H&E
eVG
Collagen 3
Collagen 4
200µm
1mm
100µm

Figure 2. The lesion in chronic human ICH consists primarily of collagen 3. Each column corresponds to the indicated patient number (for additional case and location details, see the Table and Figure S5 in the supplement). Each row is labeled by its corresponding marker. The top row provides an overview of the three chronic ICH lesions at low magnification followed by high magnification images that correspond to the dashed boxes. Van Gieson (eVG) staining at high magnification highlights (in pink) the extensive collagen found inside the lesions. Additional staining with collagen 3 and 4 reveals that the lesion consists primarily of collagen 3. All unlabeled scale bars after the first row indicate 100μm.